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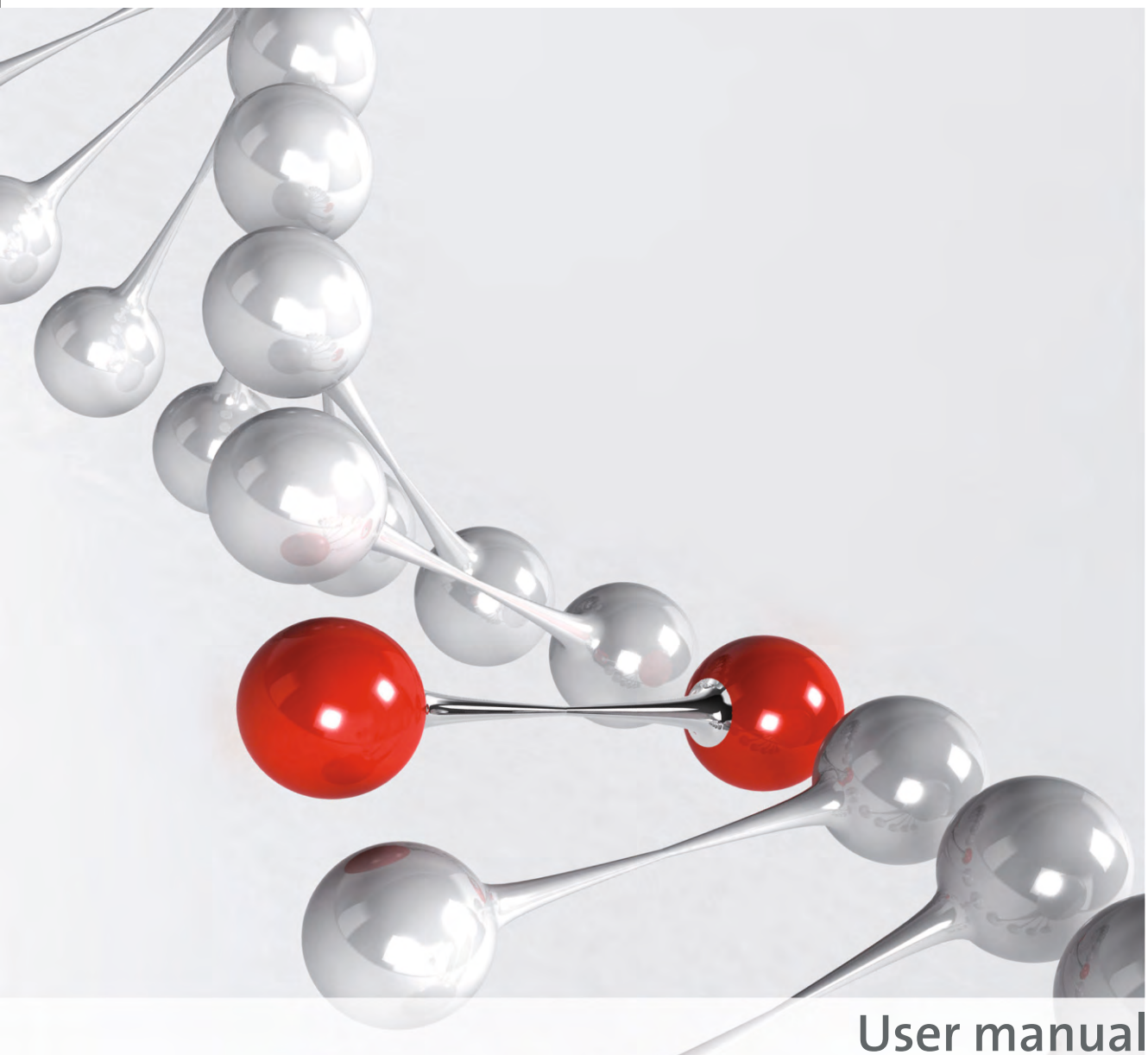

# User manual
# InviMag® Virus DNA Mini Kit/ IG
## for use on the InviGenius®, STRATEC Molecular GmbH

for automated purification of viral DNA from serum, plasma, cell-free body fluids, rinse liquid from swabs with magnetic beads

CE
IVD

REF 2442120100

STRATEC Molecular GmbH, D-13125 Berlin

# Instructions for InviMag® Virus DNA Mini Kit/ IG

The **InviMag® Virus DNA Mini Kit/ IG** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles for a very efficient and reliable isolation of viral DNA with high purity in a fully automated process using the InviGenius® platform.

The viral DNA binding magnetic particles used in the procedure are characterized by a high specific surface area, uniform size distribution and good suspension stability. Therefore, the particles are highly suitable for high throughput processing of nucleic acids.

The **InviMag® Virus DNA Mini Kit /IG** is the ideal tool for walk-away isolation and purification of viral DNA from 12 samples of fresh or frozen plasma, serum, cell-free body fluids and rinse liquid from swabs with the InviGenius® fully automated pipetting system.

The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Virus DNA Mini Kit /IG** with the InviGenius® system was intensely tested.

CE **IVD** Compliance with EU Directive 98/79/EC on *in-vitro* medical devices.

Not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on *in-vitro* medical devices is not recognized.

Trademarks: InviGenius®, Invisorb®, InviMag®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviGenius®, InviMag® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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## Kit contents of InviMag® Virus DNA Mini Kit/ IG

Store the **MAP Solution B** at 4°C!
Store lyophilized **Proteinase K/ Carrier-RNA (PKC Tube)** at 2 - 8 °C!
Store dissolved **Proteinase K/ Carrier-RNA** at –20 °C!
Store all other kit components at room temperature (RT)!

| | **8 x 12 extractions** | **reagents sufficient for** |
|---|---|---|
| **Catalogue No.** | 2442120100 | |
| **Lysis Buffer HL** | 30 ml | 8 runs |
| **PKC / IG** | for 8 x 800 µl working solution | 1 vial per run |
| **RNAse free Water** | 15 ml | |
| **Binding Buffer HL** | 8 ml<br>(final volume 32 ml) | 4 runs |
| **MAP Solution B / IG** | 2 x 2.6 ml | 1 vial per 4 run |
| **Wash Buffer I** | 60 ml<br>(final volume 120 ml) | 8 runs |
| **Wash Buffer II** | 2 x 36 ml<br>(final volume 2 x 120 ml) | 4 runs |
| **Elution Buffer M** | 30 ml | 8 runs |
| **Incubation Plate A** | 1 | 8 runs |
| **Working Plate A** | 4 | 2 runs |
| **Elution Plate E** | 1 | 8 runs |
| **Microtube Cap** | 8 | |
| **Sheath Box** | 1 (2 racks á 48 sheaths) | 4 runs per plate (48 pieces) |
| **Sealing Foils** | 4 | |
| **Incubator Stripe Foils** | 2 | |
| **Initial steps** | Add 24 ml of 99.7% Isopropanol to the **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.<br>Add 60 ml of 96-100% ethanol to the bottle **Wash Buffer I.**<br>Add 84 ml of 96-100% ethanol to each bottle **Wash Buffer II**.<br>Resuspend each **PKC tube** with 800 µl RNase-free water per tube | |

## Symbols

**Manufacturer**

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

## Storage

All buffers and kit contents of the **InviMag® Virus DNA Mini Kit /IG**, except **PKC** (Proteinase K / Carrier-RNA mixture) and **MAP Solution B** (magnetic beads) should be stored at room temperature and are stable for at least 12 months at these conditions.

**PKC Tube:** Lyophilized **PKC Tube** should be stored at 2-8°C. If dissolved in RNase-free water storage at -20°C is recommended.

**MAP Solution B:** The magnetic beads should be stored at 4°C.

**Wash Buffer II:** Wash Buffer II charged with ethanol should be stored at room temperature and should be appropriately sealed. If any precipitates are visible within the provided solutions, solve them by carefully warming up to 30°C.

**Room temperature (RT) is defined as a range from 15-30°C.**

## Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Virus DNA Mini Kit/ IG** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, the product will be replaced free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Virus DNA Mini Kit/ IG** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Virus DNA Mini Kit/ IG** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

**For technical support or further information please contact:**

**from Germany: +49-(0)30-9489-2901/ 2910**
**from abroad: +49-(0)30-9489-2907 or contact your local distributor.**

## Intended use

The **InviMag® Virus DNA Mini Kit/ IG** is designed for fully automated extraction and purification of viral DNA from up to 200 µl serum or plasma based samples. Up to 12 samples can be processed using a patented magnetic beads system and the InviGenius® robotic platform.

It is advised to provide at least 550 µl sample per tube (dead volume) to prevent pipetting distribution errors due to the liquid level detection (LLD) process. The final processed sample volume is 200 µl. The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of viral DNA from fresh or frozen sample. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 9).

Common collection tubes (not provided) and anticoagulants (EDTA and citrate, *but not heparin*) can be used to assemble a set of samples. All utilities (reagents and plastics) – except conductive tips - required for preparation of viral DNA are provided by the **InviMag® Virus DNA Mini Kit/ IG**.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

*The kit is in compliance with EU Directive 98/79/EC on in-vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in-vitro medical devices is not recognized.*

## Product use limitation

The kit is validated for viral DNA extraction from cell-free body fluids and rinsed liquids, specifically for human serum and plasma. Related applications will need a separate validation. Extraction of eukaryotic DNA from samples has not been evaluated with this kit.
The included chemicals are only useable once.
Differing of starting material may lead to inoperability. Therefore, neither a warranty nor guarantee in this case will be given, implied or expressed.
The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA’ 88 regulations in the U.S. or equivalents in other countries.
All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.
The chemicals and the plastics are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.
The product with its contents is not suitable for consumption.

## Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the waste generated by the **InviMag® Virus DNA Mini Kit/ IG** procedures for residual infectious materials. Contamination of the waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, all waste has to be considered infectious and should be handled and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Virus DNA Mini Kit/ IG** to which they apply, are listed below as follows:

**Lysis Buffer HL**

warning

H302-315-319 P280-305-351-338

**Proteinase K:**

danger

H315-319-334-335 P280-305-351-338-310-405

**Wash Buffer R1**

warning
H302-312-332-412 EUH032 P273

| | |
|---|---|
| **H302:** | Harmful if swallowed. |
| **H315:** | Causes skin irritation. |
| **H319:** | Causes serious eye irritation. |
| **H315:** | Causes skin irritation. |
| **H334:** | May cause allergy or asthma symptoms or breathing difficulties if inhaled. |
| **H335:** | May cause respiratory irritation. |
| **H312:** | Harmful in contact with skin. |
| **H332:** | Harmful if inhaled. |
| **H412:** | Harmful to aquatic life with long lasting effects. |
| **EUH032:** | Contact with acids liberates very toxic gas. |
| **P280:** | Wear protective gloves/protective clothing/eye protection/face protection. |
| **P305+P351+P338:** | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present. Continue rinsing. |
| **P310:** | Immediately call a POISON CENTER or doctor/physician. |
| **P405:** | Store locked up. |
| **P273:** | Avoid release to the environment. |

**Emergency medical information can be obtained 24 hours a day from infotrac:**

**outside of USA: 1 – 352 – 323 – 3500**
**inside of USA : 1 – 800 – 535 – 5053**

## Product characteristics of the InviMag® Virus DNA Mini Kit/ IG

The **InviMag® Virus DNA Mini Kit/ IG** is the ideal tool for an efficient and fully automated viral DNA extraction and purification of fresh or frozen samples using magnetic beads in combination with the InviGenius® system.

| Starting material | Yield | Time for preparation |
|---|---|---|
| 200 µl serum or plasma<br>200 µl cell free body fluids<br>200 µl rinsed liquid from swab | depends on the sample (source and storage) | ~ 60 min for 12 samples |

The DNA isolation process is based on the interaction of nucleic acids with silica coated magnetic particles at adapted buffer conditions. The InviGenius® instrument will automatically perform all steps of sample and reagent distribution. The DNA purification procedure is performed without any user intervention, except the initial loading of the system, thus allowing safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated by the automated purification process. The use of unique bar codes for samples and reagents avoids unwanted transpositions.

The InviGenius® instrument uses magnetic rods to transport the DNA-binding magnetic particles through the various extraction phases: lysis, binding, washing and elution. The volume of buffers and other liquids necessary for DNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique.

After a sample specific lysis - using **Lysis Buffer HL** and **PKC** - optimal binding conditions are adjusted by addition of **Binding Buffer HL.** The viral DNA binds to the simultaneously added magnetic particles and is separated from the solution by the magnetic rods controlled by the InviGenius® system. Subsequent to three washing steps of the particle bound nucleic acids, the DNA is finally eluted in **Elution Buffer M**.

Due to the high purity, the eluted viral DNA is ready-to-use in a broad panel of downstream applications such as:

- real-time PCR* (quantitative RT-PCR, like TaqMan® und LightCycler® technologies)
- or array technologies

For the isolation of DNA from a single serum, plasma or blood sample STRATEC Molecular offers the **Invisorb® Spin Virus DNA Mini Kit** or for 8–96 samples the **Invisorb® Virus DNA HTS 96 Kits** for use on a centrifuge, vacuum manifold or other robotic workstations.

**For further information please contact:** phone +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries phone +49 (0) 30 9489 2903, 2907 or ask your local distributor.

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

## Example data

To show a typical result from an extraction process, viral DNA derived from Hepatitis B (200 µl starting material) and water samples (negative control) were isolated in a cross-contamination study and analyzed by real-time qPCR on a Corbett Rotorgene cycler.

### Quantitation data for Cycling A.FAM

| No. | Colour | Name | Ct | Rep. Ct | Rep. Ct Std. Dev. |
|---|---|---|---|---|---|
| 1 | | HBV | 31.65 | 32.26 | 0.67 |
| 2 | | Water | | | |
| 3 | | HBV | 31.89 | | |
| 4 | | Water | | | |
| 5 | | HBV | 33.37 | | |
| 6 | | Water | | | |
| 7 | | HBV | 32.62 | | |
| 8 | | Water | | | |
| 9 | | HBV | 32.37 | | |
| 10 | | Water | | | |
| 11 | | HBV | 31.69 | | |
| 12 | | Water | | | |
| 13 | | NTC | | | |
| 14 | | NTC | | | |
| 17 | | PTC | 31.47 | 31.65 | 0.25 |
| 18 | | PTC | 31.83 | | |

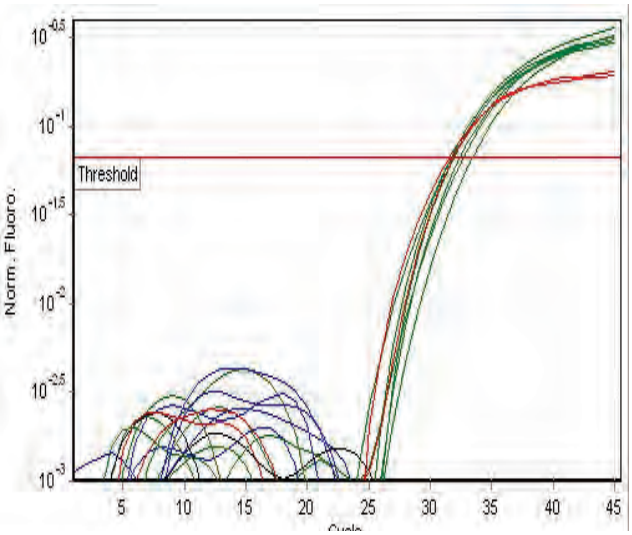



**Fig. 1: RT-qPCR from HBV samples**

For the RT-qPCR 2 µl template was used. The temperature profile was 95°C for 10s, 60°C for 30s using 45 cycles. Figure 1 demonstrates the good conformity and reproducibility of the samples (green) and the PTC (positive control, red). No amplification products for the water samples (blue) and the NTC (negative control, black) were detectable demonstrating that no cross-contamination occurs during the extraction process.

## Sampling and storage of starting material

Best results are obtained using freshly extracted samples. As long as the samples are not shock-frosted with liquid nitrogen or incubated with DNase inhibitors or denaturing reagents, the present viral DNA is not stable. DNA contained in such deep frozen samples is stable for months. However, the DNA purification should be performed as soon as possible.

**Serum and plasma**

After collection, serum and plasma derived from blood (treated with anticoagulants like EDTA or citrate, but **not** with heparin), synovial fluid samples or other cell free body fluids, swabs as well as stool samples can be stored at 2 - 8°C for short time (up to 24 h). For long-term storage, we recommend freezing samples in aliquots at –20°C. Frozen plasma or serum samples should not be thawed more than once. Multiple thawing and freezing cycles before isolating the viral DNA should be avoided. This may lead to denaturation and precipitation of proteins, resulting in reduced viral titers and yields. In addition, cryoprecipitates formed during freezing and thawing cycles can cause additional problems. If cryoprecipitates are visible, centrifuge them at app. 6.800 x g for 3 min. The cleared supernatant should be aspirated carefully without disturbing the pellet and processed immediately. This step will not reduce the viral titer.

**Swabs**

The protocol works with fresh prepared swabs as well as with dried swabs. The protocol has not been validated for isolation of viral DNA from swabs which are stored in special storage buffers of other providers.

STRATEC Molecular will not take responsibility if other sample types than described above are used or if the sample preparation advices are modified

## Principle and procedure

**Lysis**

Samples are lysed at denaturing conditions in a 2.0 ml Deep Well Plate at elevated temperatures in the presence of 200 µl **Lysis Buffer HL** and 40 µl **PKC**.

**Binding of the DNA**

After addition of 250 µl **Binding Buffer HL** and 40 µl **MAP Solution B** to the lysate, the DNA is bound to the surface of the magnetic beads.

**Removing residual contaminants**

Contaminants are efficiently removed using 600 µl **Wash Buffer I** and twice 650 µl **Wash Buffer II** while the nucleic acids remain bound to the magnetic beads.

**Elution**

The viral DNA is eluted in 100 µl **Elution Buffer M**. A defined aliquot of 50 µl is finally transferred to the **Elution Plate**. The bottom magnets guarantee a magnetic beads free eluate which is ready-to-use in different downstream applications like real-time PCR* (quantitative RT-PCR, like TaqMan® und LightCycler® technologies) or array technologies.

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

## Yield and quality of viral DNA

The amount of purified RNA in the **InviMag® Virus DNA Mini Kit/ IG** procedure depends on the sample type, the virus content, sample source, transport, storage, and age.

Yield and quality of isolated viral DNA is suitable for any molecular diagnostic detection system. The diagnostic tests should be performed accordingly to the manufacturers' specifications.

***Note:*** *If beads are visible in the eluate plate, transfer the eluate to a new reaction tube and centrifuge for 1 min at maximum speed (e.g.13.000 rpm). Using the Elution Plate E directly for centrifugation is also possible.*

Different amplification systems can vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates from this kit contain both viral DNA and Carrier-RNA. In general, the amounts of Carrier-RNA will greatly exceed the amounts of isolated viral nucleic acids.

Yields of viral DNA isolated from biological samples are normally less than 1 µg and difficult to determine photometrically due to the very low concentrations*. Keep in mind, that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the RNA present in the samples.

The kit is suitable for downstream analysis with nucleic acid techniques like qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed accordingly to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral DNA yield.

*) In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded because the kit contains Carrier-RNA. The Carrier-RNA consists of poly-A RNA fragments in size of 100-1000 base pairs. The kit is not dedicated for applications using this kind of analysis.

## Important notes

**Important points before starting a protocol**

Immediately upon arrival of the product, inspect the kit and its components as well as the package for any apparent visible damages and correct quantities. If there are any unconformities, please notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately
- Do not combine components of different kits.
- Avoid microbial contaminations of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow.
- This kit should only be used by trained personnel.

## Preparing reagents and buffers

Before starting a run, equilibrate all reagents at room temperature. Where necessary, gently mix and redissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.
**Lysis Buffer HL, MAP Solution B** and **Elution Buffer M** are ready-to-use.

| **8 x 12 viral DNA extractions** |
|---|
| Add 24 ml of 99.7% isopropanol to the **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. |
| Resuspend the lyophillized **PKC tube** by addition of 800 µl RNase-free water. Mix thoroughly. Store dissolved and unused **PKC tubes** at –20°C. |
| Add 60 ml of 96-100% ethanol to the bottle **Wash Buffer I.** Mix thoroughly and always keep the unused bottle(s) firmly closed! |
| Add 84 ml of 96-100% ethanol to each bottle **Wash Buffer II**. Mix thoroughly and always keep the unused bottle(s) firmly closed! |

*Note: Dissolved* PKC *must be stored at -20°C. Avoid multiple freezing and thawing cycles because this may lead to degraded Carrier-RNA and reduced kit performance. We recommend to prepare only as much tubes/reagent as needed for one assay run.*

## Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Conductive pipette tips (1.100 µl filter tips, order no. 501110040)
- Disposable gloves
- Distilled $H_2O$
- Vortexer
- 96-100% ethanol
- Isopropanol*

*) The **InviMag® Virus DNA Mini Kit/ IG** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

**Possible suppliers for Isopropanol:**

**Carl Roth**
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

**Applichem**
2-Propanol für die Molekularbiologie
Order no. A3928

**Sigma**
2-Propanol
Order no. 59304-1L-F

**Possible primary tubes, manufacturer, cat. no.**
- BD Vacutainer (6 ml) K2E BD 367864
- PS Tube Sarstedt (5 ml) 55.476

# Important indications

### 1. Minimum volume of samples in primary tubes

The procedure of the **InviMag® Virus DNA Mini Kit /IG** is optimized for the isolation of viral DNA from up to 200 µl cell-free body fluids (serum, plasma, CSF,) and rinsed liquids from swabs. It is advised to provide at least 550 µl sample per tube to prevent pipetting distribution errors during sample processing.

### 2. Sample volume smaller than 200 µl

For samples of a smaller volume than 200 µl please adjust the volume to 350 µl using PBS or distilled RNase/DNase free water.

### 3. Elution volume

The final elution volume is 100 µl.

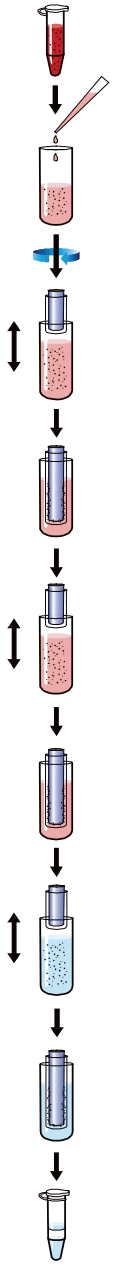
### Prevention of cross-contamination

To comply with the demanding guidelines of *in-vitro* diagnostics the pipettor of the InviGenius® is routed in such a way that possible contamination risks are minimized. However, we recommend applying the supplied well-strips and –foils beforehand and after a run on the unused (and used afterwards) wells of the Incubation and Working Plate A

# Scheme of the InviMag® Virus DNA Mini Kit/ IG

***Add the primary tubes in the sample loading rack.***

***Add the Buffers in the Buffer loading rack.***



200 µl sample is mixed with 200 µl **Lysis Buffer HL;** 40 µl **PKC**. An incubation of 15 minutes is performed at 95°C.

250 µl **Binding Buffer HL** *(follow preparing instructions)* and 40 µl **MAP Solution B** are added to the lysate

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed viral DNA with:
1 x 600 µl **Wash Buffer I**
2 x 650 µl **Wash Buffer II**

Magnetic separation

Elution of viral DNA in 100 µl **Elution Buffer M**

Magnetic separation and removal of **MAP Solution B**

Pure viral DNA

# Working Protocol: Isolation of viral DNA

**Please read the instructions carefully and conduct the prepared procedure.**

***Important Note:*** *The protocol is optimized for the isolation of viral DNA from up to 200 µl of cell-free fluid. To prevent possible distribution errors we recommend providing at least 400 µl sample in total to ensure stable processing.*

## Preparing the samples for processing on the InviGenius®-system

**1. Extraction of viral DNA from serum, plasma, cell-free body fluids**

This type of sample can be processed directly without any preparations. Please ensure to supply at least 550 µl sample or adjust the volume up to 550 µl using PBS or RNase/DNase free water.

**2. Extraction of viral DNA from swabs**

Rinse each swab with enough cooled RNase/DNase free water or cooled PBS (max. 500 µl) and incubate the swab for 5 min. Then squeeze out the swab at the inside of the tube and discard it. Finally, transfer the liquid into a sample tube and insert it into the sample rack of the InviGenius® instrument. Keep in mind that the samples have to be labelled with bar codes. Otherwise the samples have to be registered manually within the InviGenius® software during the sample loading step.

## Prevention of cross-contamination

To comply with the demanding guidelines of *in-vitro* diagnostics the pipettor of the InviGenius® is routed in such a way that possible contamination-risks are very unlikely. However, we recommend applying the supplied well-strips and –foils beforehand and after a run on the unused (and used afterwards) wells of the Incubation and Working Plate A. Take care not to seal lanes that will be required for a successful run.

# Preparing of the internal control for the InviGenius®-system

**Please read the instructions carefully and conduct the prepared procedure.**

### Using an internal control (IC)

Using the InviGenius Virus DNA Kit in combination with a commercially available amplification system may require introducing an internal control (IC) into the purification procedure to monitor the efficiency of sample preparation.

Internal control DNA or RNA (IC) must be combined with Carrier RNA stock solution (or with Carrier RNA - Proteinase K - stock solution) in one mixture. For each sample the machine transfers a volume of 40 µl of the stock PKC solution to the lysis mix.

The PKC vials contain 800 µl stock solution, so the internal control for 20 samples must be added directly to the PKC tube.

### Example – Calculation:

Per Extraction 4.5 µl of the DNA control would be needed:

4.5 µl / RXN X 20 RXN = 90 µl

→ PKC stock solution has to be made by adding 800 µl – 90 µl = 710 µl RNAse free water. Then add 90 µl control DNA followed by a mixing step.

### Notes:

If you only have indication of amount per reaction, please calculate by using eluate and template volume.

If the internal control (IC) is stable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs (e.g., armored RNA), it can alternatively be added to the sample shortly before beginning sample preparation. But consider that a bigger amount of internal control is necessary when using bigger volumes of primary sample tubes. The minimal required sample volume in the sample tube has to be at least 550 µl.

If the internal control (IC) is naked/unprotected DNA or RNA and it is unstable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs, the internal control must **not** be added directly to the samples.

Refer to the manufacturer's instructions to determine the optimal amount of internal control (IC) for specific downstream applications. Using an amount other than that recommended may lead to wrong quantification results.

## General overview of the InviGenius® System

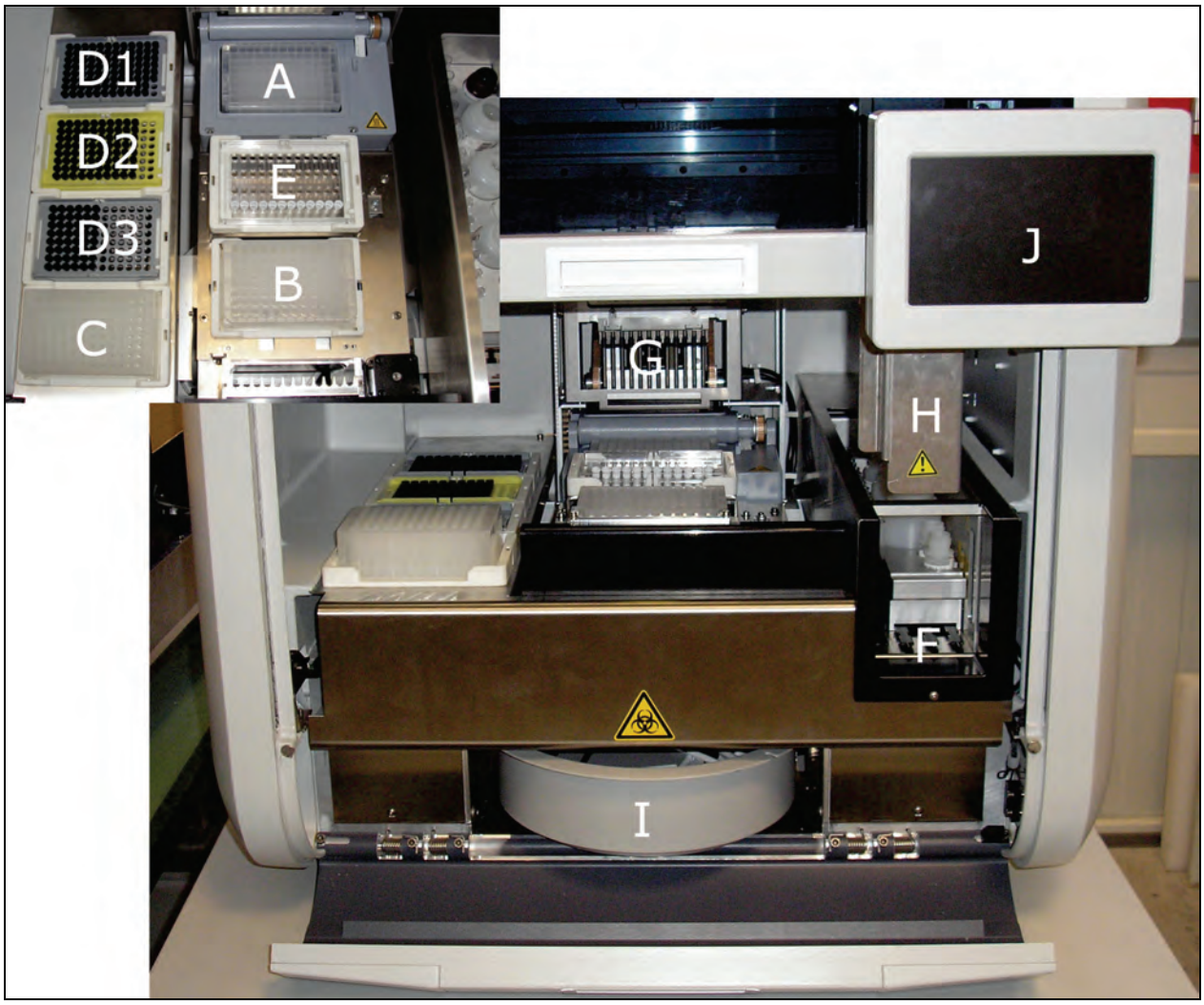



**Figure 1: Frontal view of the InviGenius®-System**

There are three plate positions available in the InviGenius® system which can be loaded with corresponding plates: the incubator k (A), the working (B), and the eluate position (C).

Lysis is performed at the incubator position (A), whereas the washing and elution process is performed at the working position (B). The eluate - containing the extracted nucleic acids – will be finally transferred to the eluate position.

Additionally, there are three loading positions available for disposable tip trays (D1-D3) and one position (E) for the disposable sheaths. The loading bay (F) is located at the very right side of the instrument. The sample rack is loaded into the far left lane whereas the reagent rack is loaded into the right lanes of the loading bay.

The Magnetic Separation Head (MSH) (G) is located on top of the incubator lid (parking position). The fully automatic pipettor (H) is installed above the loading bay (parking position). The disposable waste tray (I) is located behind the lower cover of the InviGenius®.

Interaction with the InviGenius® instrument is performed by use of the touch LCD (J) located at the top front right side.

# Preparing and loading of the InviGenius® System

### Preparing the reagents

Before you start, dissolve one vial of Proteinase K and Carrier RNA with 800 µl DNAse-free water, respectively.

### Preparing the system

Turn on the InviGenius® system using the power switch located on the right back side of the instrument. The InviGenius® software can be started by double clicking the InviGenius® icon located on the desktop. Keep the door of the InviGenius® system closed during initialization.

After initialization of the InviGenius® system a login screen appears (Figure 1).

Log-in with the provided user name and password.

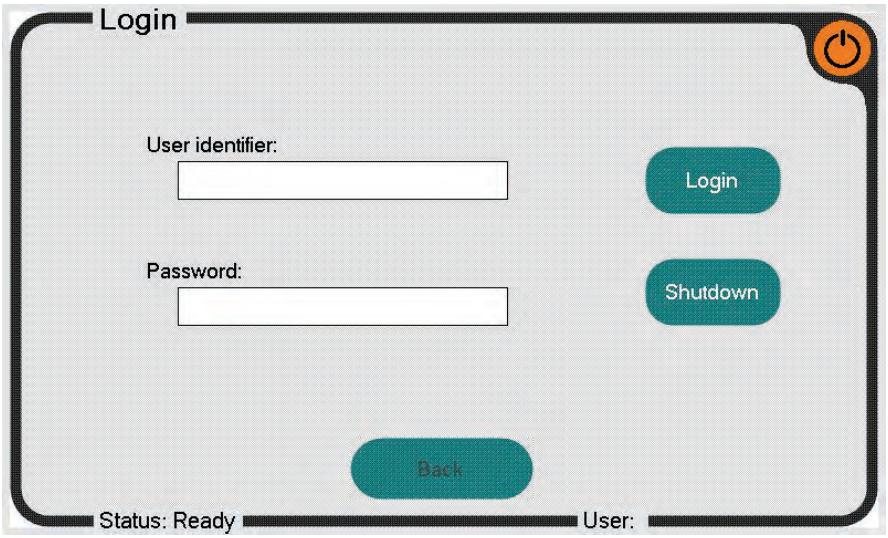



**Figure 1: Login screen of the InviGenius® software**

After login the main screen of the InviGenius® software is shown (Figure 2). Select “Loading” to start with loading of the system. Select “Processing” to define and run an assay if the system has already been loaded.

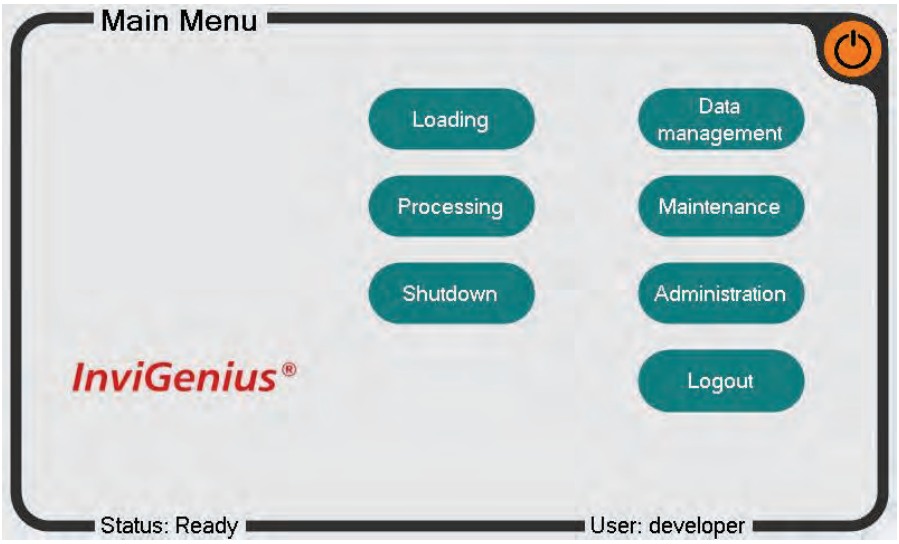



**Figure 2: Main menu of the InviGenius® software**

**Sample Loading**

After selecting “Loading” the sample loading screen appears.

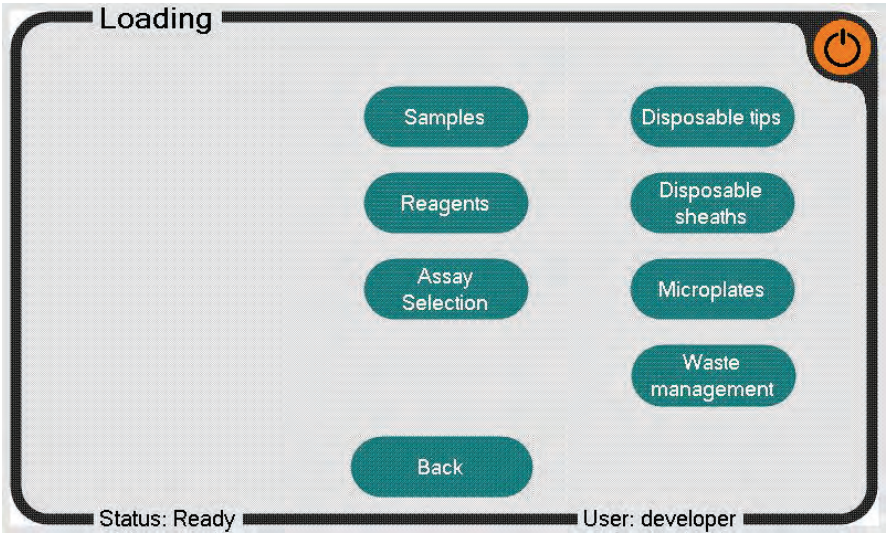



**Figure 3: Loading screen of the InviGenius® software**

Select “Samples” to proceed with the sample loading screen.

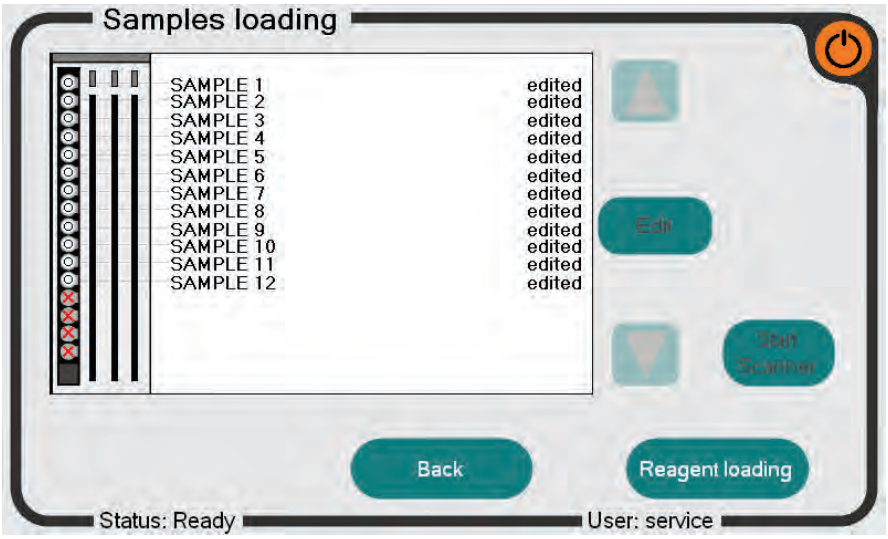



**Figure 4: “Sample-loading” screen of the InviGenius® software**

Please add the samples to the rack. Please decap the tubes before transfer to the loading rack.

If available the primary tubes should be used directly as sample tubes. If the samples are not provided in primary tubes, please prepare the sample rack with primary tubes that are prefilled with samples from which the viral DNA shall be extracted. Sample tubes are not provided with the kit and can be ordered at e.g. Sarstedt (order no. 55.476, 5 ml tubes, 75x12 mm, PS) or see recommendation at page 10, chapter: reagents and equipment to be supplied by user.

For each reaction a sample volume of 200 µl is processed. However, it is recommended that the total sample volume filled in the sample tubes should be at least 400 µl to ensure stable processing. Please take care, that only the first 12 positions of the sample rack can be processed due to the limited number of wells per row of the plastics. For correct identification of the sample tubes the unique bar codes must face to the bar code scanner located at the right side of the loading bay.

After inserting the sample rack in the very left lane of the loading bay, an updated screen will show the identifiers read from the sample bar codes (Figure 7). In case of unsuccessful

sample identification, remove the rack, check the bar code orientation, and reinsert the rack slowly. It is also possible to rename the samples by selecting the corresponding sample by using the arrow fields, followed by the “Edit” button.

After a certain time (about 5 min) the bar code scanner is inactivated. In that case the user has to restart the scanner with the “START SCANNER” button if the loading procedure is not finished.

After successful loading of the samples proceed with reagent loading by selecting “Reagent loading” on the bottom right hand side of this screen.

**Reagent Loading**

The reagent loading process is analogous to the sample loading procedure.

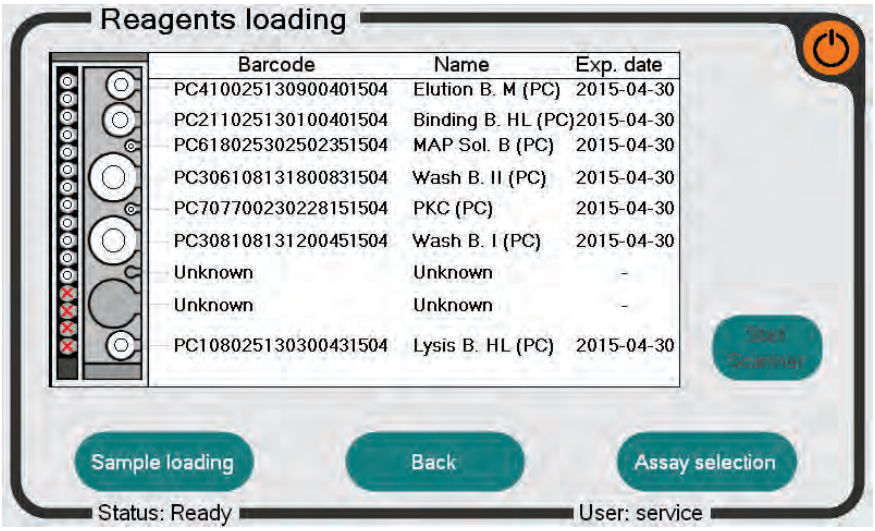



**Figure 5: “Reagent-loading” screen of the InviGenius® software**

Insert all provided reagents into the provided reagent rack of the InviGenius® system. Take care that the bar code labels face to the right side of the loading bay and decap the bottles and tubes. The order of the inserted reagents is not crucial because the type and position of a reagent is identified by the unique bar code. However, the possible loading positions are limited by the size of the used bottles.

After rack insertion the loading status of the reagents will be shown. In case of unsuccessful reagent allocation, remove the rack, check the bar code orientation and repeat the procedure slowly.

**Assay Selection**

Select the Virus DNA assay “DVIR_E100_S200” and proceed with disposable tip loading.

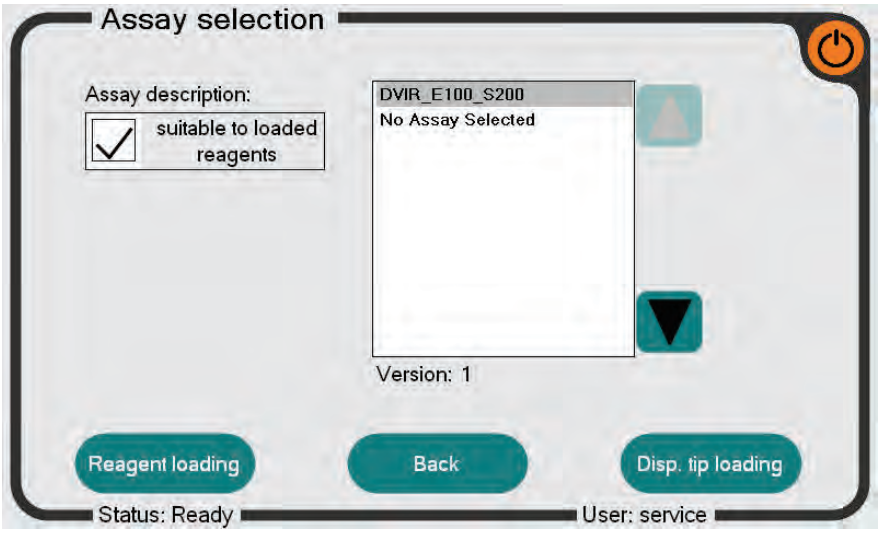



**Figure 6: “Assay-selection” screen of the InviGenius® software**

**Disposable Tip Loading**

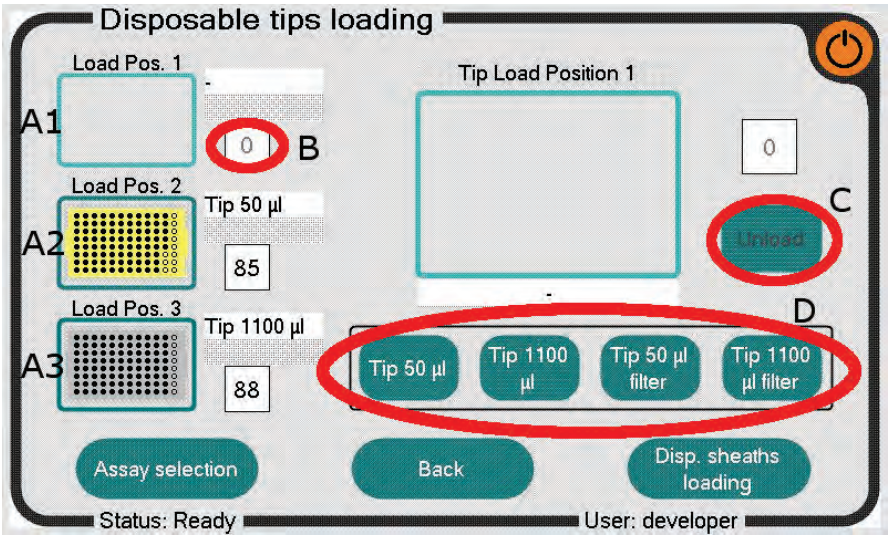



**Figure 7: Disposable tip loading screen**

There are three tip rack positions on the InviGenius® system (Fig. 10, A1-A3; corresponding to Fig. 3, D1-D3). Remaining tip-numbers are shown in B. Tip-numbers can be changed by pressing the number-field directly.

Empty tip-racks can be unloaded and reloaded by:

1.) Pressing the Loading-Position directly (The software will focus this loading position on the main screen)

2.) Pressing the Unload-Button C

3.) The loading-position can be refilled with a new tip-rack by pressing on the corresponding tip-rack on D

Each position can be filled either with 50 µl or 1100 µl filter or non-filter tips. However, for the Virus DNA assay, only 1100 µl filtered tips will be used.

***Attention:*** ***It is very important to allocate the type of tips correctly in the software that have been loaded into the instrument. In case of false tip allocation, overfilling of the tip will irreparably destroy the pipettor head!***

All protocols should be used in combination with filter tips to ensure efficient prevention of sample or reagent cross-contaminations. STRATEC Molecular will give no guarantee or responsibility if contaminations occur due to the use of non-filtered tips.

***Note:*** ***Disposable tips are not supplied within the kit. We recommend the use of validated conductive tips, which can be ordered at STRATEC Molecular. STRATEC Molecular offers 50 µl conductive tips (10x 96 pieces, order no. 5011120100) and 1100 µl conductive tips (10x 96 pieces, order no. 5011120200). Be sure that conductive tips are used otherwise the tip detection unit, installed in the pipetting unit, will reject the tips and no run will be possible.***

**Disposable Sheaths Loading**

The sheaths are used as protection devices for the magnetic rods.

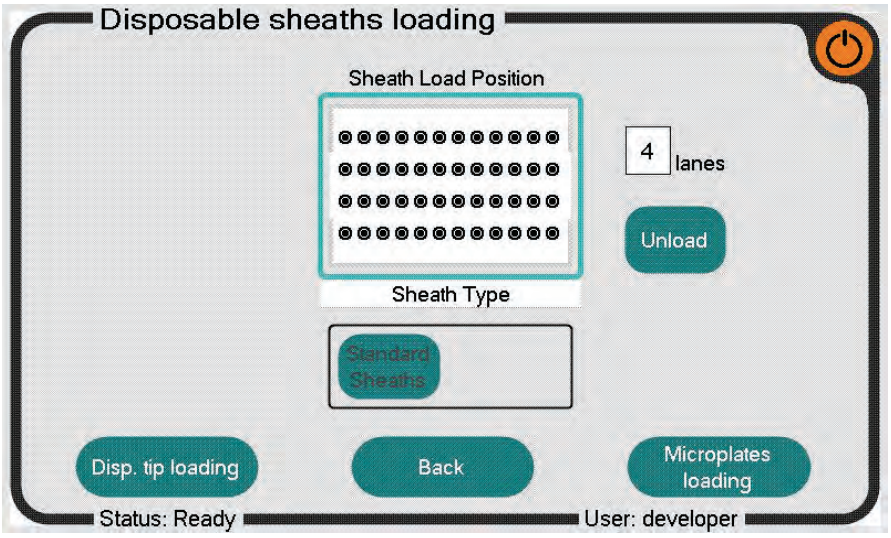



**Figure 8: Disposable sheaths loading screen**

The loading procedure of the disposable sheaths works analogous to the disposable tip loading screen. For a run, always 12 disposable sheaths (one row in the sheaths rack) are used, regardless of the processed sample numbers. This is done, to assure that the rods are always protected against contaminations.

In general, the number of sheaths supplied within the kit is sufficient for the amount of runs printed on the kit package. If you are lacking sheaths, they can be ordered separately at STRATEC Molecular (100 pieces bulk, order no. 5011120300 or 10 x 48 pieces, order no. 5011120400).

Comparable to the disposable tips loading it is possible to define the number of rows left in the tip rack by pressing on the displayed number area. Make sure that the disposable sheaths are loaded (and displayed) consistent to the manually loaded sheaths in the rack to ensure correct sheaths pick up. Don't remove single disposable sheaths within a row of the sheaths rack if less than 12 samples are processed within one run because there is a sheaths detection sensor installed in the device. If less than 12 sheaths picked up by the instrument a warning will be displayed and all picked up sheath will be discarded into the waste before a next row of sheaths will be picked up for testing.

To avoid contaminations, we strongly recommend to not wash/reuse any disposed sheaths!

### *Plate Loading*

Analogous to the previous loading screens, the incubation, working and elution plates are loaded within the plate loading screen (Figure 9).

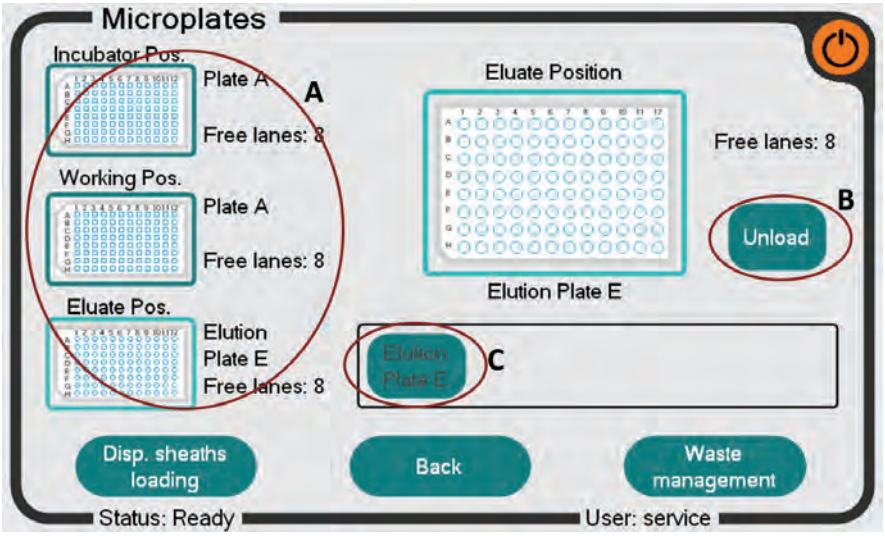



**Figure 9: Plate loading screen**

In general, the Incubation Plate A and Working Plate A (identical) are used at the incubator and working position whereas at the eluate position the Elution Plate E is used.

Used plates can be unloaded and reloaded by:

1.) Pressing the plate position directly (A). The software will focus at the plate position on the main screen.

2.) Pressing the “Unload” button (B)

3.) The plate can be reloaded by pressing on the offered plate in (C).

For a successful run the InviGenius® needs one free lane in the incubator position, seven free lanes in the working position and one free lane in the eluate position.

Please make sure that the depicted lanes on the monitor are consistent with the real lanes in the corresponding positions.

To avoid contaminations, we strongly recommend to not wash/reuse disposed plates!

**Waste management**

Please make sure that the waste tray´s capacity is sufficient for your planned assay. If not, empty the solid waste.

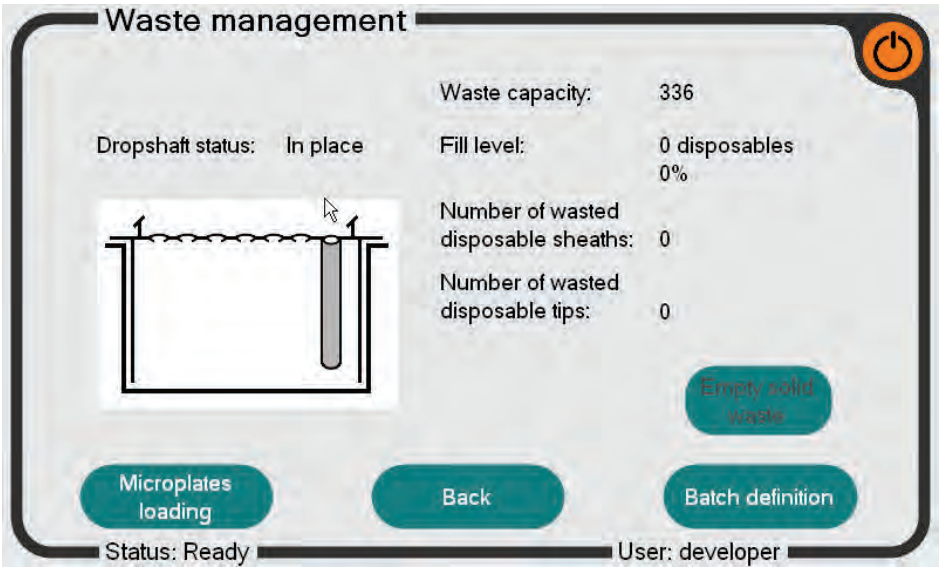



**Figure 10: Waste-management-screen**

If waste tray has been emptied, please click on the “Empty solid waste” button (A) afterwards.

**Batch definition**

Please select the appropriate assay and check the samples you want to process in this run.

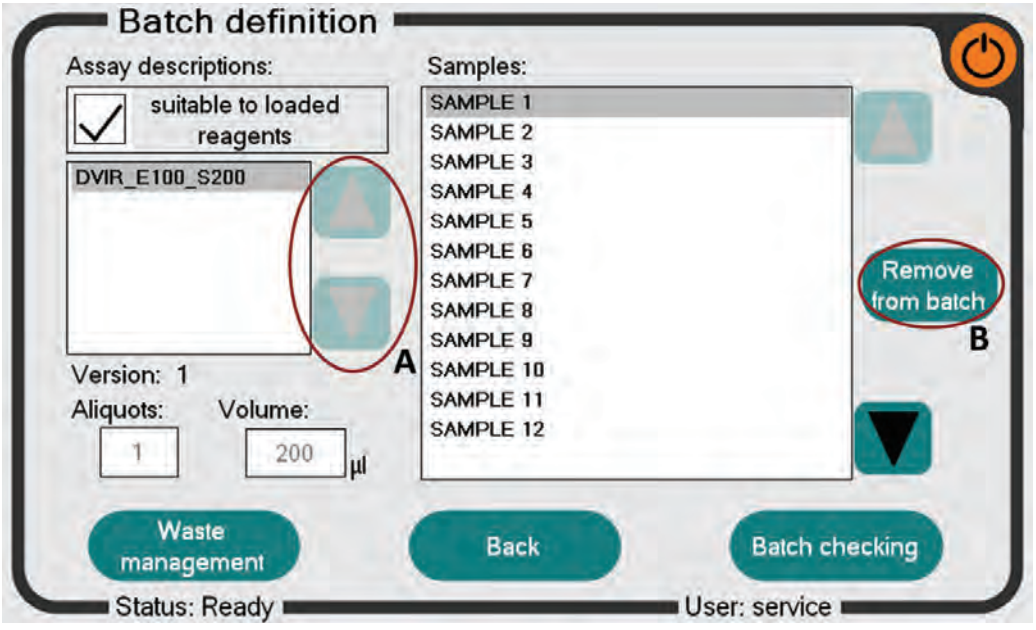



**Figure 11: Batch-definition-screen**

Please select the desired assay and recheck the allocated samples that should be processed in this run. It is possible to switch between the offered assays by using the two arrow buttons (A).

By default, all loaded samples are selected to be processed in this run. If samples have to be excluded from the batch, exclude them by selecting the corresponding sample and clicking on the “Remove from batch” button (B).

**Batch checking**

This screen shows a summary of all checked disposables, samples and reagents in one informational screen. Please make sure that all required components are loaded correctly. In case of any error, the problem will be highlighted in by a red font. If no errors during the loading steps occurred, proceed by pressing the button “Batch processing”.

To solve any error, click on the red highlighted field and follow the instructions printed on the instrument screen.

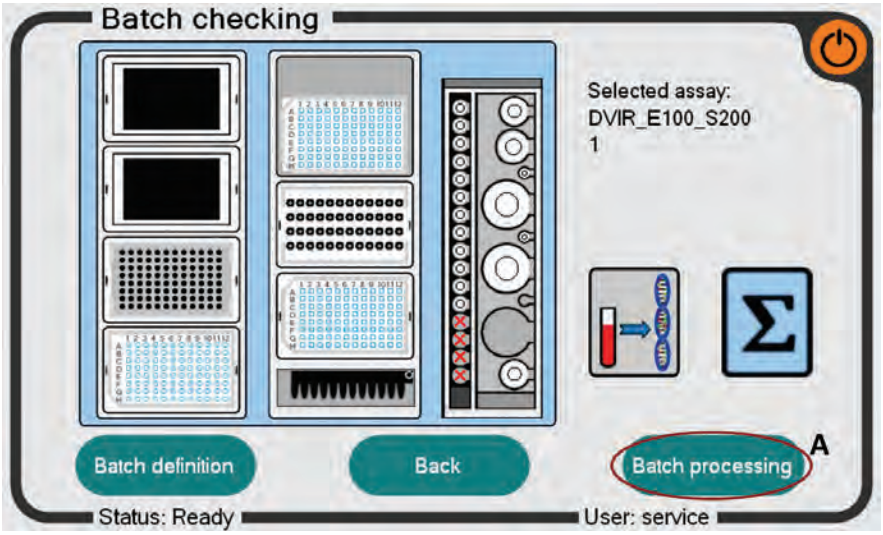



**Figure 12: Batch-checking-screen**

**Batch processing**

After closing the system-door, the assay can be started by pressing the “Start”-Button (A). The door will be locked during the run and the system will start with sample processing. The door will only be unlocked after a run has been successfully finished or if an error occurs that requires user interaction. Do not try to force open the door during a run. This will cause an abort of the run!

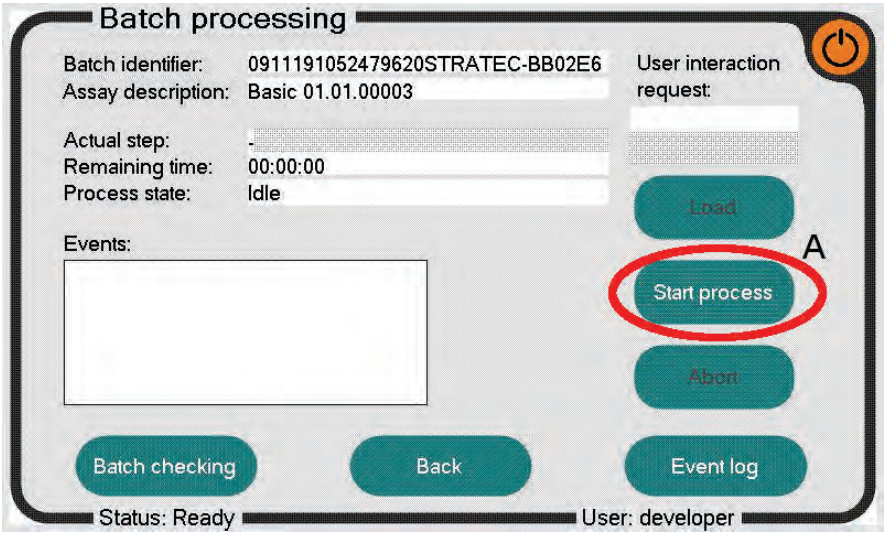



**Figure 13: Batch-processing-screen**

At the end of the process, the viral DNA containing eluate is located in the appropriate eluate position and can be used for further applications.

***Note:*** *The complete process will take approximately 60 minutes.*

**After a run**

After a run is completed and no additional run shall be started, unload all plates and reagents and store them according to GLP guidelines. Please keep in mind, that the plates could contain infectious material.

As with all medical/clinical and diagnostically equipment, all waste (liquids, tips, sheaths and plates) should be treated as potentially dangerous bio-hazard waste.

## Daily maintenance (UV decontamination)

The InviGenius® system is equipped with an internal UV lamp (254 nm wavelength) that should be used daily either at the end of the working day or in the morning before a run is started. The suggested decontamination time is about 20 min. To start the UV decontamination go to the main menu of the InviGenius software and select “Maintenance”.

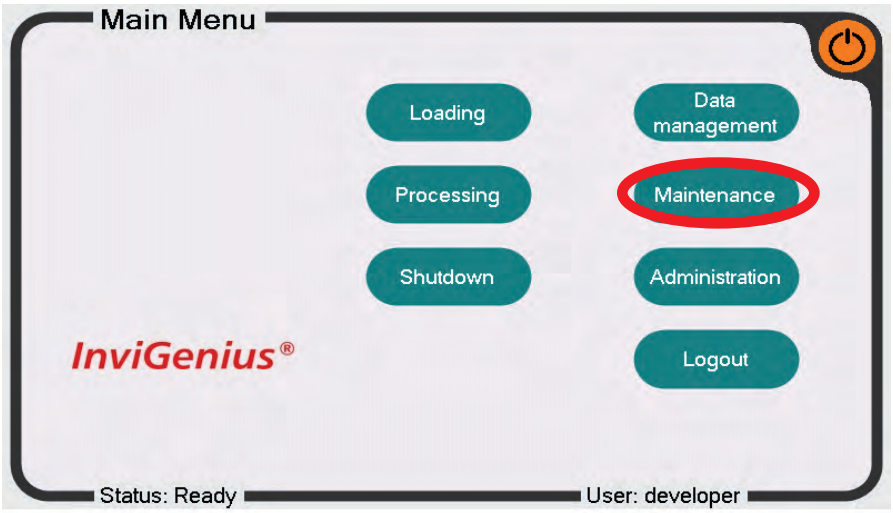



**Figure 14:** Main screen of the InviGenius® software

When the sub item “Maintenance” is opened, select “UV decontamination”

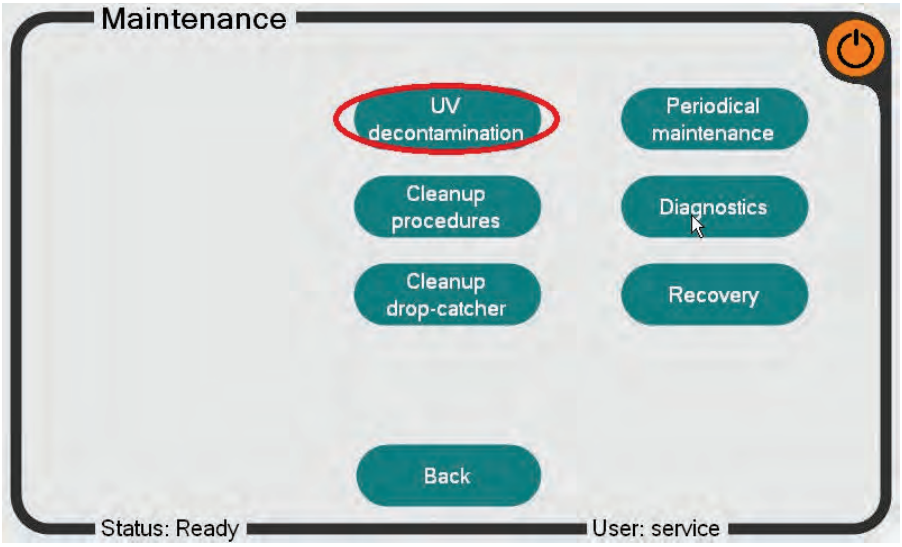



**Figure 15:** Maintenance screen of the InviGenius® software

In the UV decontamination menu adjust the exposure time (A) and finally press the “Start” button (B). During the decontamination process the instrument door will be locked to prevent any UV radiation release in the lab.

**Warning:** ***UV radiation is harmful. It causes serious burns of the skin and leads to irreparable damage of the eyes and skin. Ensure that no lab personnel is submitted to direct UV light. Do not try to force open the instrument door during the decontamination process.***

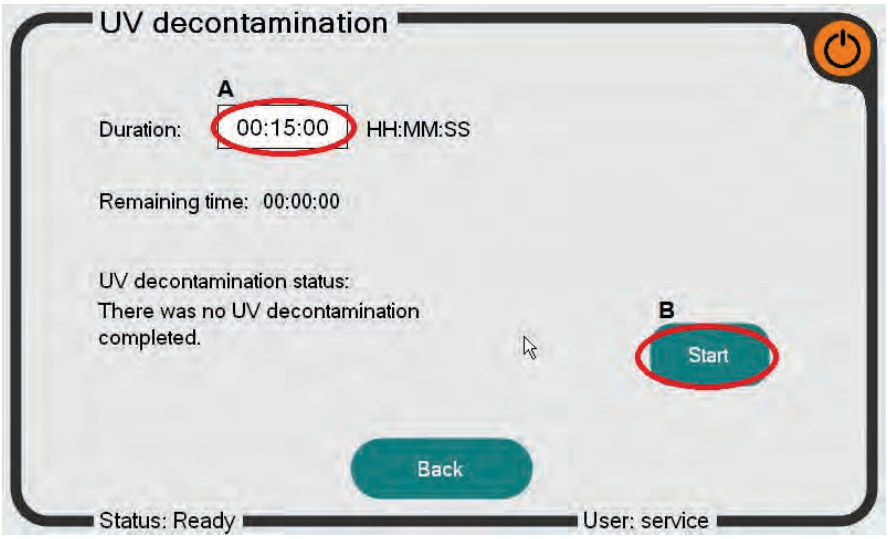



**Figure 16**: UV decontamination screen

When the decontamination is finished, go back to the main menu by using the “Back” button. The device is now decontaminated and can be either switched off or used for sample processing. We recommend decontaminating the instrument daily.

## Troubleshooting

| Problem | Probable cause | Comments and suggestions |
|---|---|---|
| pipetting distribution errors | pipetting of **PKC** failed | ensure that the lyophilized **PKC** is lyophilized with the appropriate volume of water before usage |
| | samples transfer failed / incomplete | the sample tube must contain at least 400 µl sample |
| | reagent / buffer transfer failed / incomplete | ensure that the supplied **Wash Buffers / Binding Solution** is filled up properly with either ethanol or isopropanol<br>do not reuse bottles more often than described in Tab.1 because they will be rejected by the system |
| low concentration of extracted DNA | blood components settled | in case of large sample volumes (>>1 ml) carefully premix the sample tube before inserting it into the sample rack |
| | no/ too much ethanol added to **Wash Buffers** | ensure that the **Wash Buffers** have been filled up properly with ethanol as indicated in Tab. 1 |
| degraded or sheared DNA | incorrect storage of starting material | ensure that the storage of starting material is correct<br>avoid multiple freezing and thawing cycles of the material |
| | old material | ensure that the starting material is fresh or stored at appropriate conditions (for long time storage at –20°C)!<br>avoid multiple thawing and freezing cycles of the material<br>old material may contain degraded DNA |
| no assay selectable | combination of reagents from different kits / missing required reagent | make sure that only and all reagents belonging to one kit type are used. a combination of reagents belonging to different kit types is not supported |
| eluted DNA is brownish colored | Residual magnetic particles are left in eluate | centrifuge the eluate plate at full speed for 1 min and transfer supernatant to a new plate / tube |

# Ordering information

| Product | Package Size | Catalogue No. |
|---|---|---|
| InviMag® Virus DNA Mini Kit/ IG | 8 x 12 preparations | 2442120100 |

| Related Products | Package Size | Catalogue No. |
|---|---|---|
| Invisorb® Spin Virus RNA Mini Kit | 50 preparations | 1040300200 |
| Invisorb® Spin Virus RNA Mini Kit | 250 preparations | 1040300300 |
| Invisorb® Spin Virus DNA Mini Kit | 50 preparations | 1040200200 |
| Invisorb® Spin Virus DNA Mini Kit | 250 preparations | 1040200300 |

**InviGenius® and consumables**

| | | |
|---|---|---|
| InviGenius® | 1 unit | 5011100000 |
| Starting Box I/ IG<br>Sheath Box<br>Conductive filter tips, 1 ml; 2 x 2 rack/ pack (384 pieces)<br>5 Waste Trays<br>120 sample tubes | 1 box | 2400110100 |
| Sheath Bundle | 10 x 48 pieces | 5011100300 |
| Sheaths | 1000 pieces | 5011100200 |
| Conductive filter tips, 1 ml | 10 x 96 pieces | 5011100400 |
| Waste tray/ IG | 25 pieces | 5011100100 |
| InviGenius® | 1 unit | 5011100000 |

## Possible suppliers for Isopropanol

**Carl Roth**
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

**Applichem**
2-Propanol für die Molekularbiologie
Order no. A3928

**Sigma**
2-Propanol
Order no. 59304-1L-F

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